

trifugal forces on flowing particles are also less in magnitude than those due to viscous drag ($F_{\text{drag}} \sim 0.1\text{--}0.4\text{ nN}$).

[0213] Based on this preliminary analysis that neglects particle wakes and interactions with the flow field, it appears that the dominant force responsible for biasing particular stable positions is viscous drag due to the Dean flow. Additionally, particles with density less and greater than the suspending fluid would experience centrifugal forces in opposite directions (Δm is of opposite sign) and not lead to focusing to a single stream. This further suggests that Dean flow-induced viscous drag is the controlling force. An asymmetric channel may function as shown in FIG. 4. Here the viscous drag along the midline of the channel is larger, leading to directional bias, whereas particles already within the potential minimum, due to the superposed inertial lift forces, remain trapped. These particles cannot escape because of less viscous drag on the particle in the region where the two vortices split or rejoin.

Example 2

[0214] Particles within the exemplary channel geometries described herein can be ordered and focused with extreme precision and with stability, as shown in FIGS. 17A and 17B. In particular, the stability of the focused streams of particles over time is assayed to demonstrate the utility of the phenomenon for focusing in flow cytometer and coulter counter systems. The stability and precision of inertially focused streams can be characterized by imaging a solution of 10- μm polystyrene particles over 10 minutes of continuous flow at $R_p = 0.24$. In the example shown in FIGS. 17A and 17B, each image had an exposure time of 700 ms, sampling an average of 1,100 passing particles. In FIG. 17A, intensity profiles are obtained from each stream and a Gaussian fit is made to this profile. There are two parameters involved: the center position of the Gaussian fit and the full width at half maximum extracted and plotted for each time point. In FIG. 17B, these two parameters are plotted for each point on the same axis. The average full width at half maximum of the focused stream was 5% larger than the average particle full width at half maximum imaged on the same microscope system. The standard deviation of the center position of the focused stream was determined to be 80 nm in the y direction, and the focused stream's average width was only 1.05 times the average width of a single particle. Although other external forces, such as magnetic, optical, and dielectrophoretic, can also be used to bias a particular equilibrium positions within the rectangular flow field, an approach using hydrodynamic forces with a curved channel structure may be ideal. The additional forces increase with the flow rate, and only a minor geometric change is required to focus particles, with no additional mechanical or electrical parts.

Example 3

[0215] FIGS. 18A-18D illustrate that in addition to the focusing of particles across the transverse plane of the channel, self-ordering of particles in the longitudinal direction, along the flow lines can also occur. High-speed imaging (2- μs exposure) can be used to reveal characteristic long trains of particles (10-15 particles) with uniform spacing that alternate between the four stable lateral positions in rectangular channels, as shown in FIGS. 18A and 18B, or are concentrated in a single stream for asymmetric channels, as shown in FIGS. 18C and 18D. In particular, FIGS. 18A and 18C represent 10 μm diameter particles in a flow rate of $R_c = 120$. As shown in

FIG. 18A, trains of particles tend to alternate between positions instead of occupying several coincidentally. FIGS. 18B and 18D represent autocorrelation functions (ACF) that confirm particle ordering with an average distance of 36 μm in the straight channel and an average distance of 48 μm in the curved channel.

[0216] As illustrated by the above embodiment, particle-particle distances below a threshold are not favored, and self-ordering in a longitudinal direction results. A shorter preferred distance is observed at higher R_c in rectangular channels than in asymmetric curved channels, as shown in FIGS. 18B and 18D. Ordered particle trains described herein are comparable to macroscale systems, where it has been postulated that preferred distances may arise from the interaction of secondary flows around rotating particles in a shear flow. In this case, the detached secondary flow itself may act as an object. For example, rigid particles ($\sim 0.5\text{-mm}$ diameter) in large ($\sim 1\text{-cm}$ diameter) cylindrical tubes will form long trains above $R_c \sim 450$ with stable interparticle spacing decreasing with R_p . In the systems described herein, robust ordering occurs for a lower $R_c \sim 90$.

Example 4

[0217] In another embodiment, additional particle ordering and alignment can be observed with reference to FIGS. 19A-20B. Self-ordering for cells in diluted (2% vol/vol) whole blood occurs as for particles in buffer solutions, as shown in FIG. 19A. Deformable particles such as cells may experience additional hydrodynamic forces in the applied flow field; however, from the experimental results whole blood, droplets, and cultured cells were found to behave as rigid particles in straight and curving microchannels. FIG. 19B illustrates a segment of a peak plot obtained from the image by data convolution with a kernel representing the in-focus particle. Intensity here represents the level of fit to an in-plane particle. Images at a rate of $\sim 15,000$ cells per second were obtained in this system. Using a time series extracted from consecutive images, the particles flowing through the detection volume can be counted and analyzed as shown in FIG. 19C. A histogram of distances between particles is plotted demonstrating the limit on particle spacing that allows easy analysis (5% of particles are spaced closer than 16 μm apart).

[0218] A fourth dimension of axial rotational alignment in asymmetric particles can also occur within the channels described herein. FIG. 20A illustrates a spatial map of red blood cells flowing through the detector area over 20 ms. The rotational, axial, and focal alignment of the cells can be seen more clearly in magnification. Here, red blood cells are aligned with the disc face in the plane of the image. In particular, discoid red blood cells aligned rotationally such that the disk axis was parallel to the rectangular channel wall, as can be seen in most clearly in 20B.

Example 5

[0219] Referring now to FIGS. 21-22, various levels of focusing for cells and particles of different sizes are provided as applications in separation stem directly from the differential focusing of particles of different sizes. A range of particle diameters (2-17 μm) and channel sizes ($D_h = 10\text{--}87\text{ }\mu\text{m}$) were tested over a range of $R_c = 0.075\text{--}225$ for curving asymmetric channels. The focusing results were plotted as a function of D_c and the ratio a/D_h , as shown in FIG. 21. In particular, as shown in the key above the plotted results of FIG. 21, no